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PRELIMINARY REPORT OF VITALITY AND ACTIVITY OF SPERM CELLS AND ARTIFICIAL INSEMINATION OF THE CHICKEN

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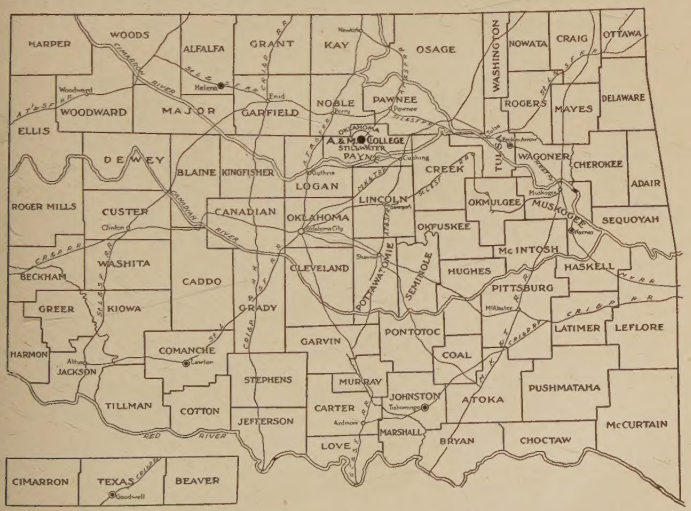

LOCATION OF THE A. & M. COLLEGE AND THE SIX SCHOOLS OF AGRICULTURE

STILLWATER, OKLAHOMA

VITALITY AND ACTIVITY OF SPERM CELLS AND ARTIFICIAL INSEMINATION OF THE CHICKEN

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The question of determining vitality in poultry is one that interests every breeder and fancier. Constitutional vigor is the first requisite of a male bird if he is to prove a good breeder. This characteristic is usually recognized by the appearance, action and general conformation of the individual; however, these qualities are not always satisfactory, since food, as well as general care and management, has such an influence on the prepotency of the male. There is also a variation in the degree of activity of normal functions within the same individual. Science has proved that the ordinary functions of the body are unexpectedly irregular. All organs of the body work better some days than others. It is only occasionally that the body is as a whole at its maximum. The organs rest in this way. There is more of a natural, distinct, periodicity in domestic fowls than in quadrupeds. This may be attributed to their general conformation and nervous temperament. Then, with all of these factors to affect the vitality, one cannot always depend on the external features as insuring the highest type of breeding qualities.

The present method of testing vitality is to allow the male bird free use of the breeding pen with the desired number of hens for a week or ten days, then to incubate the eggs which are gathered from these pens, and after one week of incubation test them for fertility. This system requires at least two weeks to determine whether or not a male has high vitality, and it always necessitates the ownership of the individual to make a test. While this practice has been in general use for hundreds of years, and the poultry industry has advanced most remarkably fast in the last decade, yet we realize the greatest possibilities have not been reached.

As a matter of investigating the question of vitality and reproduction in fowls, an experiment was outlined and a preliminary report of the results secured follows:

Object

The two leading objects of this work were: First, to study the vitality of the sperm cells of fowls under both laboratory and natural conditions; secondly, to test the possibility of artificial insemination of poultry.

Plan of the Experiment

1. To examine with a microscope the vitality and life period of the spermatozoa when kept at body temperature (106° F.) and at 34° F.

2. To determine the time required for sperm cells to pass from the cloaca to the infundibulum of the oviduct.

3. To investigate how long the sperm cells will live in the oviduct under natural conditions.

4. To attempt to fertilize sterile females with diluted and undiluted seminal fluid.

Method of Investigation

1. A sample of fertilizing fluid secured from a female after copulation was diluted with a physiological salt solution and divided into equal parts, one sample being placed in an incubator at 106° F. and the other in an ice box at 34° F.

2. These samples were examined under a microscope at intervals of from two to five hours and notes made as to the motility of cells.

3. Pullets which had never been with a male bird were killed and a microscopic examination for sperm cells made of scrapings from the oviduct at certain periods after breeding.

4. Both diluted and undiluted quantities of seminal fluid were injected into the cloaca of the female and a fertility test was made of all eggs laid after the second day.

Manner of Securing Sperm Cells

The only way to get this fluid from the male bird, so far as we know now, is by use of a rubber ladle or round-bowled spoon with a long handle. During copulation this instrument may be held under the ejaculatory ducts, but such a practice can be used only with very gentle fowls. A more successful way is to dip a sherbet spoon into the cloaca immediately after the hen has been treaded. The fluid is very white and mucilaginous.

The amount secured from one service will vary from only a drop or two to half a spoonful.

Cell Characteristics

The movement of these cells when first secured is similar to that of active "wiggletails". The head of the spermatozoon of the fowls, unlike that of larger animals, is long and piercing, with a nucleus in the anterior end, and no line of demarkation is visible between the head and body portion of the cell until it is stained. The tail measures from twelve to fifteen times the length of the body, and it cannot be traced to the posterior extremity under the microscope

thout staining. It is easy to understand why the cell should have is long, piercing shape when we know the method of fertilization hich takes place in the female. The functional oviduct in a normal n when extended measures about .66 meter (26 inches) in length m the cloaca to the funnel or infundibulum. The seminal fluid is posited in the cloaca and each cell must propel itself the entire gth of the oviduct where the two cells unite.

Life Period of Cells Remarkable

Under artificial conditions the cells did not appear active after enty-eight hours when kept at body temperature, but we know at this life period extends for days in the natural state. A number tests were carried on where virgin pullets were allowed one service the male, after which all eggs were incubated and examined for rtility. Fertile eggs were found until the fourteenth day, with the ception of a few which had escaped fertilization. Professor Roy H. Waite of the Maryland Station secured fertile eggs twenty-one days ter the male had been removed from the flock.

We are almost forced to adopt the theory that ova are not fer-ized until they leave the follicle of the ovaries and enter the oviduct, r, as soon as the male and female cells unite, fertilization is com-ete and development starts. This growth continues to such an ex-nt that a newly laid egg when broken can in many cases be identi-ed as fertile or infertile by observing the size of the blastoderm or erm. This development being apparently the same in all fresh eggs ads us to believe that this union occurs at about the same place for ch egg, since it requires from eighteen to thirty hours for it to pass wn the oviduct. If the sperm cells passed beyond the oviduct and rtilized the ova before they were developed and ready to pass from e ovaries, growth would begin at once and the germs would vary in ze according to the period of time they remained in the body cavity. or example, if fertilization should occur as much as ten days before e egg was laid, we might expect a half-developed chick when the gg passed from the uterus. But as this is not the case, cells must re-ain active in the region of the infundibulum until the ovum passes om its follicle and is "swallowed up" by the oviduct where uniting of e two cells takes place.

It was interesting to note the difference of the length of life at e high and low temperatures; those at 34° F. living from two to ree times as long as the ones at body temperature. There was also great variation in motility of cells from different males. In five ales tested the life period of cells varied in the low temperature om twenty to fifty hours.

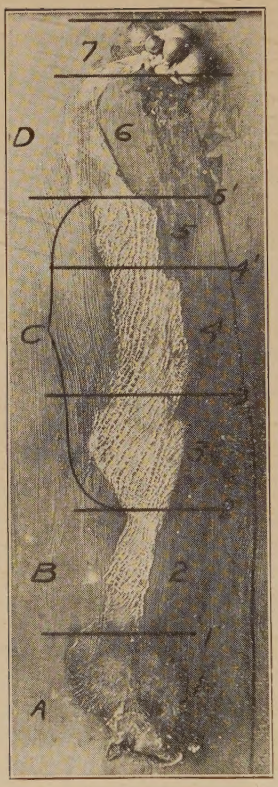

Figure 1
This illustrates the interior surface of the oviduct as it is divided into zones. 1, Zone, including all of the cloaca and uterus. 2, Second zone or isthmus. 3, 4 and 5 represent corresponding zones which comprise the section in which the albumin is secreted. 6 Is the last zone, and represents the section where cells are most numerous, and where fertilization is supposed to take place. 7, Ova

A—Uterus
B—Isthmus
C—Section in which albumin is secreted
D—Infundibulum

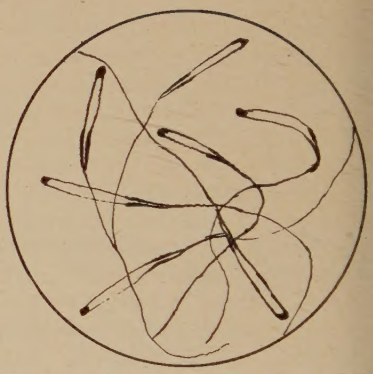

Figure 2—Drawing of the Sperm Cells of a Chicken, greatly enlarged.

The number of cells per cu. mm. from five cock birds of approxiately the same age was as follows:

No. 1, 5,470,000,
No. 2, 2,147,000,
No. 3, 3,168,000,
No. 4, 1,920,000,
No. 5, 1,936,000.

Estimates were made of the number of cells ejaculated at one ice, and a conservative number would be 500,000,000. Roosters ill tread hens from twenty to forty times daily, therefore, they can e used to a much greater advantage than they are under the present ay methods.

Rapidity With Which Cells Travel

A normal, functional oviduct was spread full length on a board, hotographed and marked off into six zones, No. 1 including all of he uterus or shell gland; No. 2, comprising all of the isthmus; Nos. 3, and 5 the region in which the albumin is secreted; and the sixth zone cluded the extreme upper portion of the oviduct.

Pullets known to be unfertlized were bred, then killed at intervals anging from one-half hour to eight weeks. The oviduct was removed s quickly as possible and scrapings from the different zones examed under the microscope to determine how far the cells had proressed. The accompanying table shows results of this investigation:

Record of Activity of Sperm Cells in the Oviduct

Leg Band No.	Hours After Breeding	Zone Found In	Numerous	Remarks.
438	½	1*	Not Very	Eggs in uterus—no shell on.
448	½	2	Not Very	Egg passing from 3d to 2d zone in oviduct.
441	½	2	Not Very	Oviduct functional; no egg in it.
367	1½	5*	Not Very	Full formed egg in the uterus.
440	1½	5*	Yes	Oviduct functional.
443	4 days	6	Yes	Oviduct functional, but empty.
442	6 days	6	Not Very	Oviduct functional, but empty.
368	7 days	1 to 6	Yes	Egg in uterus, cells very active in Zone 6.
358	14 days	5 to 6	Yes	Functional oviduct.
365	21 days	6	Yes	Functional; egg in uterus; cells apparently motile.
371	21 days	6	Not Very	Functional; ripe ova in Zone 6.
363	21 days	6	Not Very	Functional; egg in uterus.
369	28 days	1 to 6	Yes	Functional; egg in Zone 4.
446	35 days	2 to 6	Not Very	Functional; egg in uterus.
437	56 days	6	Yes	Oviduct functional.

After the fourteenth day, cells in the oviduct appeared to have reatly degenerated; that is, they were only about one-tenth of their riginal size—practically all of the tail portion being absent. From e fourteenth to the fifty-sixth day the movement in cells was the

NOTE.—1 One prime, indicates that cells were on the border of the next gher zone.

same, whether or not this motility was from life within the cell c brownian movement we have not been able to determine. How ever, the cells had all appearance of life, but with experiments a this Station fertile eggs were not laid more than sixteen days afte the males were removed. Other Stations have found an occasiona egg as long as twenty-two and twenty-six days.*

One of the most interesting facts this chart reveals is that sperm cells pass very quickly from the cloaca to the infundibulum, or fu length of the oviduct. Their progress was apparently as rapid whe there was an egg in the uterus or shell gland as when there was no Microscopic examination of the interior lining of the oviduct show numerous epithelial cells, which are covered with a vibrating mass c cilia; these no doubt aid materially in the forward progress of th reproductive cells.

Artificial Fertilization

Most of the work up to this time has been spent in studying th sperm cells of the male, the physical characteristics, activity, lon gevity and their actions after entering the oviduct. It was necessar that these things be understood before artificial fertilization could b practiced with any degree of success.

The question was, whether or not eggs could be fertilized b seminal fluid transferred from one female to another. If that could b done successfully, could a sample be diluted with a physiological sa solution and injected into the cloaca of several hens with equal su cess. From our knowledge of the life period of these cells and th number of them passed at one service, this latter method would ap pear very practical. And, if it did prove successful, there would b nothing to prevent a man who possessed a valuable male bird f stand his bird, as in stud breeding. The semen might be sent by ma and the receiver could treat a large number of his hens at a sma expense. Poultry, unlike other animals, have no periods of heat, s they could be treated any time samples may be received.

In order to test this out several hens known to be laying inferti eggs were treated in the manner suggested above with undiluted flui and a fair percent of the eggs were fertile. These eggs were inc bated and chicks hatched from them early in the fall of 1913.

This phase of the work will be investigated and tried out furth so that a complete report may be issued later.

Much credit for this report is due Dr. L. L. Lewis for supplyin all of the necessary equipment for this work and for giving valuab suggestions from time to time. Acknowledgment is also given Professor A. F. Rolf for suggestions in the preparation of this repo

*Bulletin No. 57, page 93, Maryland Agricultural College. Bulletin No. page 40, Missouri State Poultry Experiment Station, Mountain Grove, Missouri.